# Comparison of hydrocarbon yields in SA-2269 cotton grown in four test plots in Texas and Utah

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## ABSTRACT

Cotton accession, SA-2269, was grown in test plots at College Station, TX, Lubbock, TX, Oslo, TX and Hurricane, UT in 2017 to compare the environmental effects on leaf biomass, % yield of hydrocarbons (HC), and total HC (g HC /g leaves) under natural growth conditions. Very highly significant differences in g dry weight (DW) 10 leaves, % yield HC and g HC/ g DW 10 leaves were found among the test plots. Leaf biomass and g HC/ g DM 10 leaves declined as expected in the drier plots. However, the arid plot at Hurricane, UT, in the northeast Mojave Desert, had significantly larger % yield HC (8.03%), supporting the theory that drought stress can induce the synthesis of chemicals in cotton. Published on-line www.phytologia.org *Phytologia 100(4): 199-205 (Dec 21, 2018)*. ISSN 030319430.

**KEY WORDS**: Cotton, *Gossypium* spp., yields of hexane extractable leaf hydrocarbons, petrochemicals, liquid fuels.

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During routine screening of cotton accessions (Adams et al. 2017a), five high HC yielding accessions were discovered in the summer of 2016 in a seed production plot at USDA, College Station, TX (SA-1166, 13.73%; SA-1419, 13.23%; SA-1181, 12.32%; SA-3348, 11.34%; SA-2269, 11.09%).

To analyze genetic relationships among these accessions, 597 SSR bands (Hinze et al., 2016) from the 30 accessions screened for HC were used in Principal Coordinate Analysis (PCoA). The analysis showed the accessions were divided into *G. barbadense* and *G. hirsutum* (Fig. 1, left and right). The *G. barbadense* samples (8) are all improved accessions. The samples of *G. hirsutum* contain both wild and improved accessions forming a very loose group, but the wild accessions are mostly found in the upper-right quadrant of the ordination (Fig. 1). Mapping the high HC yielding accessions onto the PCoA ordination (Fig. 1) reveals they are clearly clustered in a tightly grouped set of improved accessions (Fig.

1, dashed oval). Plotting the high and highest yielding samples revealed that the three samples representing the top 13% in HC yield (SA-1181, SA-1403, SA-2269) and the highest yielding sample (SA-1419, top 3%) are found in that group (Fig. 1, dashed oval). Adams et al. (2017a) noted that the discovery of the highest yielding samples in a group of improved accessions was surprising, in view of the selection for increased cotton seed and fiber yields. Accessions selected for improved agronomic yield were not expected to also have increased HC yields.

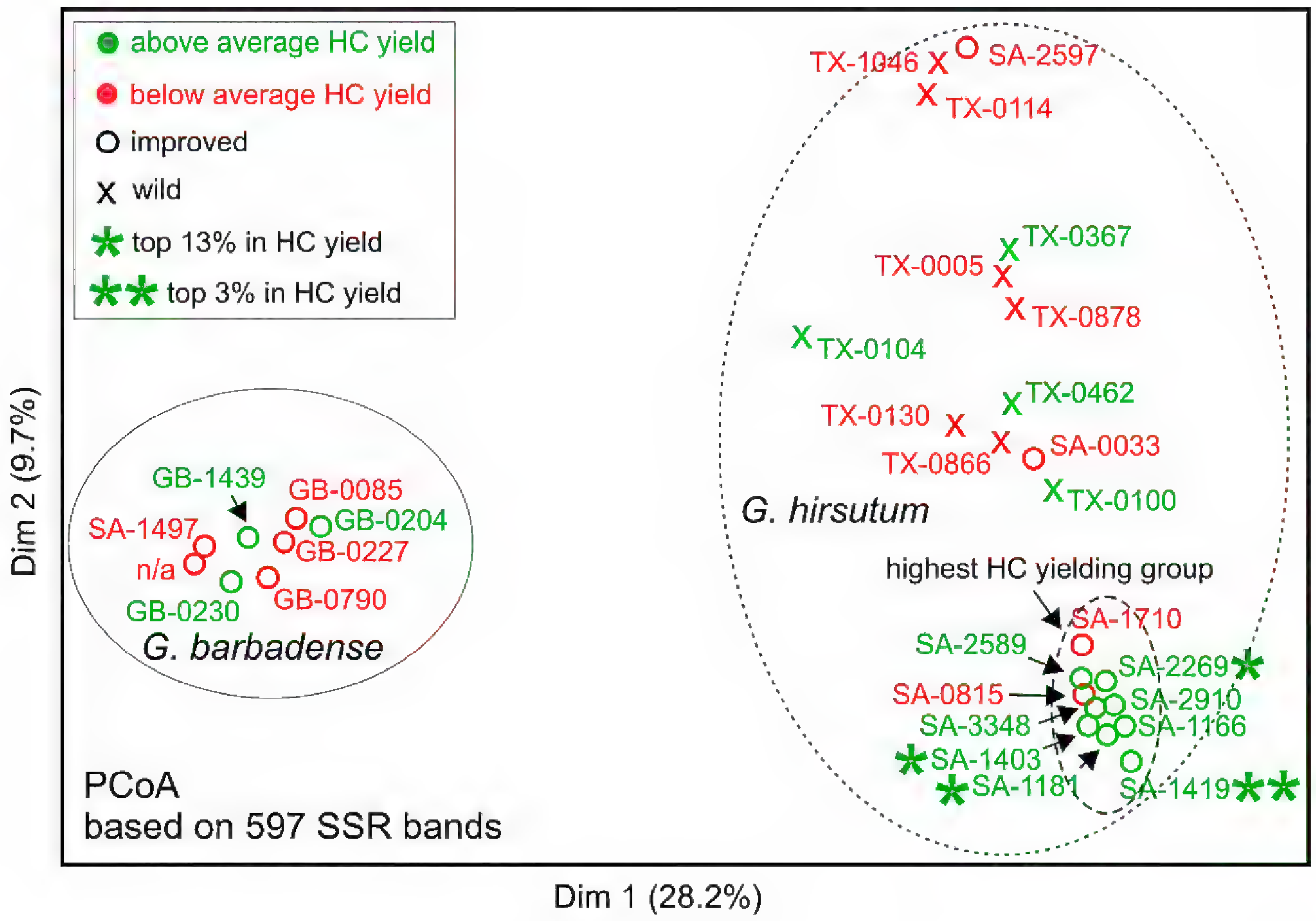


Figure 1. Principal Coordinate Analysis (PCoA) based on 597 SSR bands. The percent of variance accounted for among accessions is given on Dim 1 and Dim 2. From Adams et al. (2017a).

Adams et al. (2018a) expanded the survey by analyzing 26 additional accessions chosen from those that clustered nearest the "highest HC yielding" group (see Hinze et al. 2016). Surprisingly, %HC yields for nearly all 26 accessions (bottom, Fig. 2) had lower yields (<7.35%) than all but 7 of the 30 accessions sampled in 2016 (top, Fig. 2). It seems unlikely that more high yielding accessions were not discovered.

The question 'Are the high HC yields genetic or environmentally controlled?' led to follow-up studies (Adams et al. 2017a, b, 2018b) in which SA-2269 was grown in various environmental conditions. Table 1 shows that dry leaf weights were similar in all the situations. Percent HC yield was very high (11.09%) at College Station in 2016, but in the next test plot (2017), it dropped to 5.97%, and lower in greenhouse plants, 4.5%. g HC/ g 10 leaves mirrored the % HC yield, being nearly twice as high as when grown in other environments.

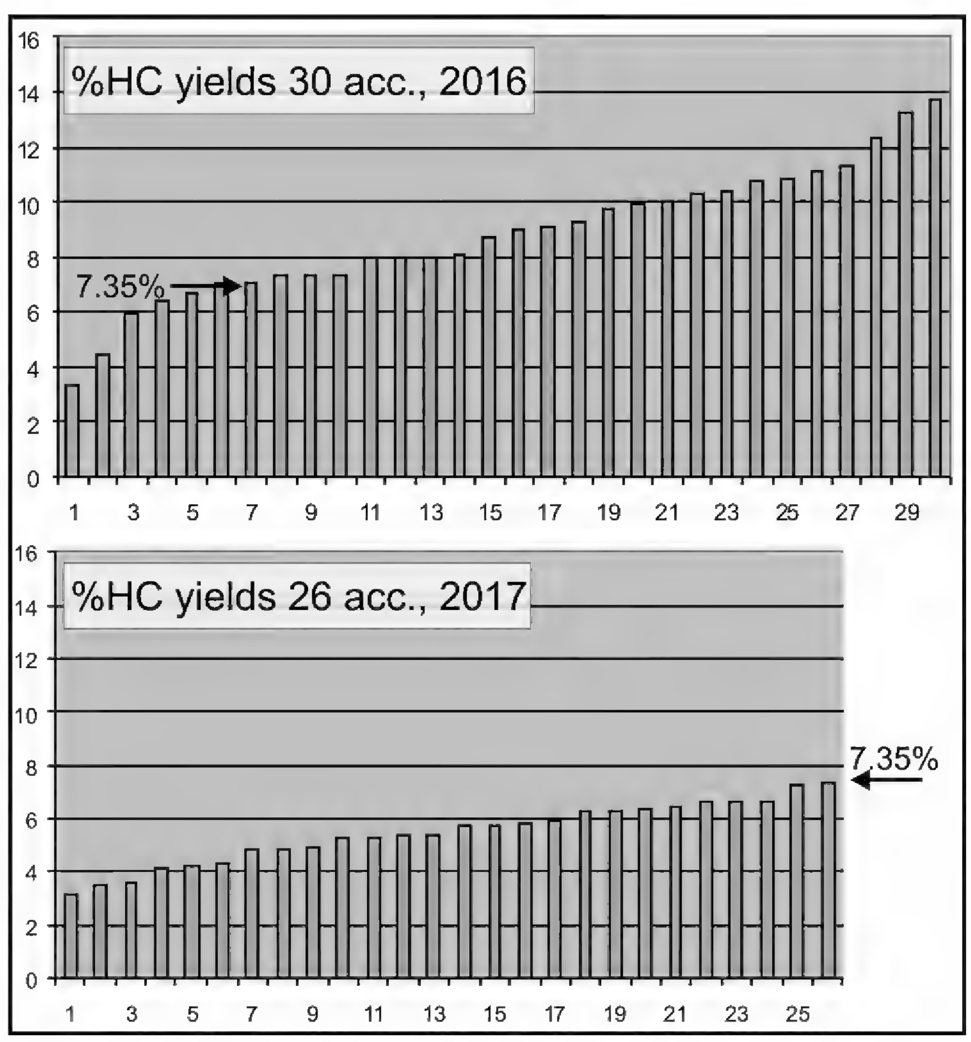


Figure 2. Histograms of % HC yields: 30 acc. (2016) (top); and 26 acc. (2017) (bottom).

Table 1. Comparison % HC yields, and g HC/ g DW 10 leaves from accession SA-2269 grown under ambient conditions at College Station, TX (2016, 2017), and grown in the greenhouse, Lubbock, TX (data from Adams et al. 2017a,b, 2018).

| SA-2269, variable | Field, College Station, TX, 2016 | Field, College Station, TX, 2017 | Greenhouse, Lubbock, TX, 2016-2107 |
|---|---|---|---|
| wt. 10 leaves | 12.44 | 13.61 | 11.93 |
| % HC yield | 11.09% | 5.97% | 4.5% |
| g HC/ g DW 10 leaves | 1.38 | 0.82 | 0.537 |

It seems that an unusual environmental incident occurred in the test plot at College Station in 2016. In a seminal paper, Stipanovic, Bell and Benedict (1999) reviewed the defensive role of pigment gland constituents in cotton. Cotton gland constituents (sesquiterpenoids, gossypol, and gossypol derivates, etc.) are a constitutive defense resource for cotton resistance to insects and diseases. Stipanovic, Bell and Benedict (1999) also discussed that these gland constituents can be rapidly synthesized in response to pathogens. Chen (2008) discusses that some constitutive chemicals may be increased to even higher levels after insect attack.

Opitz, Kunert and Gershenzon (2008) examined the response of stored (constitutive) terpenoids in cotton subjected to mechanical damage, herbivory and jasmonic acid treatments. They found that terpenoid levels increased successively from control to mechanical damage, herbivory, and jasmonic acid treatments.

The purpose of this paper is to report on changes in HC production in field cultivated cotton grown under ambient conditions in four environments: College Station, TX; Lubbock, TX; Oslo, TX and Hurricane, UT.

## MATERIALS AND METHODS

Plant Materials:
Accession SA-2269 from the U.S. National Cotton Germplasm Collection.

2017 Environments:
*College Station, TX*
Cultivated at the USDA-ARS Southern Plains Agricultural Research Center, College Station, TX, 30° 37' 5.00" N, 96° 21' 50" W, 354 ft., subsurface drip irrigation, sandy soil, annual rainfall 40". The lowest growing, non-yellowed, mature leaf was collected at random, from each of 10 cotton plants.

*Lubbock, TX*
Cultivated at the USDA-ARS Plant Stress and Germplasm Development Research Center, Lubbock, TX, 33° 35' 36.3" N, 101° 54' 4.2" W, 3243 ft., light, sandy soil, avg. annual rainfall 19.2", water was applied during the growing season to attain germination and limited growth to reflect plant water stress responses, similar to dryland production, otherwise the plants were watered only by natural rainfall. The lowest growing, non-yellowed, mature leaf was collected at random, from each of 10 cotton plants.

*Hurricane, UT*
Cultivated in a garden plot on the Scow Family Farm, Hurricane, UT, 37° 10' 10.72" N, 113° 19' 14.0" W, 3277 ft., sandy, gravely soil, avg. annual rainfall 12-16". The ten (10) lowest growing, non-yellowed mature leaves were collected at random from each of 10 cotton plants, at 1st open flower stage.

*Oslo, TX*
Cultivated in garden plot, JP TeBeest Farm, 36° 25' 0.6" N, 101° 32' 17.3" W, 3258 ft., on dryland, dark, loam soil, Oslo, TX, avg. annual rainfall, 19.3". The ten (10) lowest growing, non-yellowed mature leaves were collected at random from each of 10 cotton plants, at 1st open flower stage.

Processing Leaf Samples:
Leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained. Leaves were ground in a coffee mill (1mm). Three grams of air-dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

The shaker-hexane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the hexane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with hexane for 8 hrs. The soxhlet correction factor (sCF) was determined to be 1.14. All shaker extraction yields were corrected to oven dry weight (ODW) by multiplication of 1.085. Thus, the total CF was 1.24 (1.14 x 1.08).

ANOVA and SNK (Student-Newman-Keuls multi-range tests) were performed in program SNK (by RPA) as formulated in Steel and Torrie (1960).

## RESULTS

Biomass, hydrocarbon (HC) yields, g HC/ g 10 leaves from SA-2269 plants grown at College Station, TX; Lubbock; TX; Oslo, TX and Hurricane, UT are shown in Table 2.

Table 2. Leaf biomass, hexane extractable hydrocarbon (HC) yields, and g HC/ g DW 10 leaves from accession SA-2269 grown in the summer, 2017 at College Station, TX, Lubbock, TX, Oslo, TX and Hurricane, UT. Any data values that share the same superscript are not significantly different at P=0.05 by SNK multi-range tests. Significance: *=0.05; **=0.01; ***>0.001.

| SA-2269 grown in 2017 | College Station, TX | Lubbock, TX | Oslo, TX | Hurricane, UT | F, Significance. |
|---|---|---|---|---|---|
| g DW 10 leaves | $13.61^{A}$ | $8.48^{B}$ | $5.41^{C}$ | $1.91^{D}$ | F=166.5<br>$P=0.21 \times 10^{-8}$*** |
| % HC yield | $5.97^{B}$ | $3.95^{C}$ | $4.13^{C}$ | $8.03^{A}$ | F=34.5<br>$P=0.40 \times 10^{-6}$*** |
| g HC/ g DW 10 leaves | $0.82^{A}$ | $0.31^{B}$ | $0.23^{BC}$ | $0.15^{C}$ | F=64.1<br>$P=0.35 \times 10^{-7}$*** |

Dry leaf weights declined (Fig. 3) from the more mesic plot (College Station) to more arid plots, reaching a minimum in the driest, harshest plot (Hurricane, UT). % yields HC declined (Fig. 3) in Lubbock and Oslo in the Texas panhandle, but increased, highly significantly, in the harshest site at Hurricane, UT. g HC/ g 10 leaves was highest (Fig. 3) in the College Station plot, then declined, mirroring the leaf biomass.

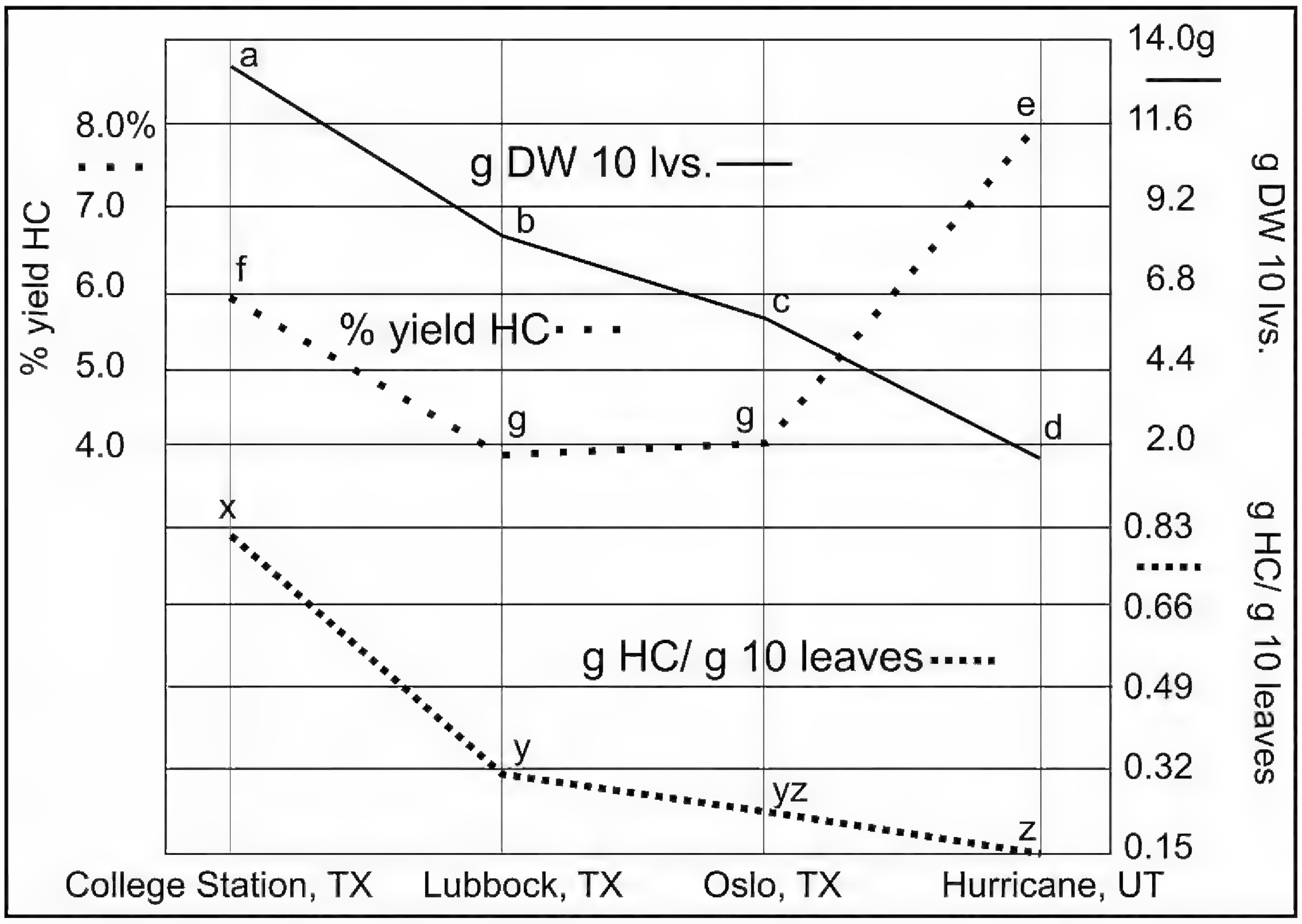


Figure 3. Graphs of leaf biomass (g DW 10 leaves), % yield HC, and g HC/ g DW 10 leaves from accession SA-2269 grown in the summer, 2017 at College Station, TX, Lubbock, TX, Oslo, TX and Hurricane, UT. Any data values that share the same superscript are not significantly differ at P=0.05 by SNK multi-range tests.

This study found very highly significant differences in g DW 10 leaves, % yield HC and g HC/ g DW 10 leaves among the test plots. Leaf biomass and g HC/ g DM 10 leaves declined as expected in the drier plots. However, there was (Fig. 3) a very high, significantly ($P=0.40 \times 10^{-6***}$) larger % HC yield in the arid Hurricane, UT plot.

The plot at Hurricane, UT is in the northeast region of the Mojave Desert at 3277 ft elevation. The seed of SA-2269 were planted on 6 June, 2017. Three plants produced their first flower on 1 Aug, then others flowered until 17 Aug. Plant leaves were harvested when a plant produced its first flower. This is a semi-arid to arid site. 2017 monthly records for Hurricane show (rainfall, avg. high temp., max. high temp.) as: June: 0.0", 100.3°F, 111.0°F; July 1.75", 100.6°F, 113.0°F; Aug 1.44", 97.2°F, 106.0°F; Sep 0.39", 88.0°F, 104.0°F. The plants were watered only as needed for survival.

This study revealed that extreme drought stress can induce the higher synthesis of free HC in cotton accession, SA-2269. However, the trade-off is the lack of biomass production under extreme drought stress. Notice that the g HC/ leaf biomass is very miniscule in the Hurricane, extreme plot. Thus, the actual effect on stress at Hurricane produced lower amount of HC. Nevertheless, study of the bio-induction of free HC in cotton seems a profitable subject for further research.

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